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Triglyceride Mobilization in the Germinating Wheat Grain

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The central role of gibberellins in initiating the mobilization of carbohydrate reserves in the germinating barley grain has been unambiguously demonstrated (see Briggs, 1971), and investigations with wheat have indicated that in this cereal the situation is similar (Eastwood, Tavener & Laidman, 1969a). Besides the major carbohydrate reserves of the starchy endosperm, however, the wheat grain also contains appreciable reserves of triglycerides in all of its tissues. Chemical analysis of the wheat bran reveals that, in this part of the grain at least, triglycerides are a major reserve material (Bechtel, Geddes & Gilles, 1964). Electron micrographs of the aleurone cells from the wheat bran (A. J. Colborne, G. Morris & D. L. Laidman, unpublished work) show large numbers of membrane-bound fat-bodies arranged peripherally around the aleurone bodies within the cytoplasm. During germination these triglycerides, and those of the starchy endosperm, are metabolized, and it is important to know whether or not this metabolism is initiated by the same hormonal system as that for the mobilization of the carbohydrate reserves. In this laboratory we have used incubated excised tissues from the wheat grain to investigate the situation (Tavener &

Laidman, 1968, 1969; Eastwood, Tavener & Laidman, 1969b).

In the quiescent grain lipase activity was not detectable in either the starchy endosperm or the bran tissues. Within 12h of the commencement of germination, however, lipase activity appeared in the starchy endosperm and soon afterwards in the bran. The activity in each tissue rose to a maximum by the sixth day of germination. Concomitant with the appearance of lipase activity in these tissues, triglyceride metabolism commenced and the triglyceride reserves were progressively mobilized throughout the experimental period of 8 days.

When endosperm halves (de-embryonated grain) were incubated alone no lipase activity appeared in the starchy endosperm and no triglyceride metabolism occurred. In the bran, however, an unexpected pattern of triglyceride metabolism occurred. In this tissue no lipase activity was detected, but some 22% of the tissue reserves of triglycerides were metabolized within the first day though no metabolism occurred thereafter. The nature of this non-lipase triglyceride metabolism has not yet been elucidated. Extracts of quiescent embryos and diffusates from germinated embryos were effective in inducing lipase activities in both the starchy endosperm and the bran tissues of the incubated endosperm halves. The same extracts and diffusates initiated triglyceride metabolism in the starchy endosperm and extended the metabolism in the bran, so that the patterns of metabolism in each tissue now resembled those occurring in the tissues of the whole germinating grain. These results demonstrate the major role played by the embryo in initiating and controlling the mobilization of triglyceride reserves in the endosperm tissues.

The effects of embryo extracts and diffusates on the incubating endosperm halves could not be simulated by any of the authentic phytohormones, either singly or in combination. On the other hand, a number of nitrogen-containing compounds at substrate concentrations were very effective in inducing lipase activity in the starchy endosperm of the endosperm halves. Most notable among these compounds were glutamine and hydroxylamine. The induced lipase activity was accompanied by the metabolism of triglyceride in that tissue, suggesting a cause-and-effect relationship between the two.

In contrast with the situation in the starchy endosperm, neither hydroxylamine nor glutamine induced lipase activity in the bran of the endosperm halves. Lipase activity in this tissue could, however, be induced by a mixture of glutamine at substrate concentration and indol-3-ylacetic acid at hormonal concentration. The same components together also induced a pattern of triglyceride metabolism similar to that occurring in the bran of germinating grain.

Gibberellic acid, abscisic acid or cytokinins could not substitute for the indol-3-ylacetic acid. The efficacy of authentic exogenous glutamine and indol-3-ylacetic acid in substituting for the embryo in inducing both lipase activity and triglyceride metabolism in the storage tissues of wheat grain indicates that these substrates might also be responsible for the induction of triglyceride mobilization *in vivo* during germination. The proof of this point must await the isolation of indol-3-ylacetic acid from germinating wheat. It is known, however, that wheat produces considerable quantities of glutamine during germination (Chibnall, 1939), and, perhaps significantly, indol-3-ylacetic acid has been detected in the grain of *Zea mays* (Powell, 1964).

The metabolism of the triglycerides in the bran of the wheat was investigated further by incubating the bran in the absence of the starchy endosperm. Under these conditions no triglyceride metabolism occurred, but the addition to the medium of either an aqueous extract of the starchy endosperm or authentic cytokinins at hormonal concentrations induced the metabolism of 22% of the bran triglyceride in a pattern identical with that in the bran of incubated endosperm halves. These results suggest that a cytokinin from the endosperm is responsible for the mobilization *in vivo* of a part of the triglyceride reserves in the bran. The suggestion is supported by the known occurrence of a cytokinin in the endosperm of *Zea mays* (Letham & Miller, 1965), but the hormone has not yet been isolated from wheat.

With regard to the nature of the induction processes, the inductions of lipase activities by glutamine and indol-3-ylacetic acid were strongly inhibited by proflavin, actinomycin D, cycloheximide and puromycin. This indicates that both RNA and protein synthesis are required for the inductions. The induction by cytokinin of the non-lipase triglyceride metabolism in the bran was strongly inhibited by proflavin, cycloheximide and puromycin, but not by actinomycin D, indicating a requirement for protein synthesis but possibly not for RNA synthesis.

In summary, it is proposed that the induction of triglyceride mobilization in the reserve tissues of the germinating wheat grain is controlled by the embryo. The hormonal induction processes involved are, however, distinct from that which induces the mobilization of reserve carbohydrates in the same species.

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An Examination of the Evidence for Stable Messenger Ribonucleic Acid in Seed

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It has been suggested that, in the early stages of the germination of certain seeds, protein synthesis is directed by a long-lived mRNA. The question whether such a long-lived mRNA is present in seed is important in considering the mechanism of the initiation of germination. The identification of mRNA presents a number of difficulties and it is the purpose of this communication to examine in detail the evidence for long-lived mRNA in seed.

Actinomycin D, which is known to interfere with transcription (Reich, Franklin, Shatkin & Tatum, 1962), has been reported to be without effect on protein synthesis in some seed (Dure & Waters, 1965; Waters & Dure, 1966; Marcus & Feeley, 1964a; Marcus, 1969; Chen, Sarid & Katchalski, 1968a), but there is some disagreement on the effects of this drug on enzyme development (Gientka-Rychter & Cherry, 1968). The conclusion that the absence of effect on protein synthesis is due to the process being independent of transcription is open to the criticism that the drug may fail to be taken up by the tissue, as was observed by Neumann (1964) and Abdul-Baki (1969). In the experiments of Chen *et al.* (1968a) it was shown that actinomycin D is taken up by wheat seed, but the possibility remains that it does not penetrate to the site of RNA synthesis. Doubt is also cast on the conclusion by the possibility, suggested by Chrispeels & Varner (1967), that synthesis of RNA may take place before the drug becomes effective.

The presence of polyribosomes in ungerminated seed, which would suggest the existence of stable mRNA, has not been established unambiguously (Marcus & Feeley, 1965; Jachymczyk & Cherry, 1968; Barker & Rieber, 1967) and, although it is well established (Marcus, 1969) that polyribosomes are formed in the early stages of germination, it has not been demonstrated whether they are formed from

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